PHD PROJECT DESCRIPTION
(4000 characters max., including the aims and work plan to de published online)

Project title: *Development of analytical procedures for the extraction, separation, and determination of antisense oligonucleotides and siRNA used for the treatment of genetic diseases*

1.1. Project goals

- synthesis, characterization, and application of new materials for extraction and separation;
- testing various chromatographic modes (exclusion chromatography, ion-exchange chromatography, hydrophilic interaction chromatography, ion-pair chromatography) and detectors (UV, fluorescent, and mass spectrometer) for oligonucleotide analysis;
- application of different extraction methods for isolation of antisense oligonucleotides and siRNA from biological samples.

1.2. Outline

Genetic diseases are caused by inherited mutations that disrupt normal gene structure or expression, often leading to severe, progressive disorders with limited treatment options. For many years, therapy for genetic diseases focused primarily on symptomatic management rather than targeting the underlying molecular defects. Recent advances in nucleic acid–based therapeutics have transformed this landscape, with antisense oligonucleotides (ASOs) and small interfering RNA (siRNA) emerging as powerful approaches for treating genetic diseases. These strategies act at the RNA level, enabling selective modulation of gene expression through mechanisms such as splicing correction, transcript degradation, or inhibition of protein translation.

ASOs are short, single-stranded, chemically modified oligonucleotides that bind complementary RNA sequences. They can induce RNase-mediated degradation or alter pre-mRNA splicing. ASO therapies are often administered intrathecally to the central nervous system. siRNA therapeutics utilize the endogenous RNA interference pathway to promote sequence-specific degradation of target mRNA via the RNA-induced silencing complex. These agents are typically administered systemically and require specialized delivery systems to ensure stability and tissue targeting. As development in this field continues, ASO- and siRNA-based therapies represent an increasingly important class of precision treatments for genetic diseases.

Given that breakthroughs in ASO and siRNA therapies began only a few years ago and drugs have been in use for a relatively short time, extensive research is needed to accurately determine the fate of drugs in the body and link it to treatment effectiveness. However, such studies require appropriate tools and methods. Traditional methods used for the separation and determination of ASO and siRNA have some disadvantages: poor sensitivity (nanomolar levels), lengthy hybridization, low dynamic range, and low signal amplification. Thus, new strategies that focus on improving the specificity and sensitivity of oligonucleotides, DNA, and reducing the time are essential. The most promising tool appears to be liquid chromatography coupled with mass spectrometry (LC-MS). The development should focus on synthesizing and applying stationary phases with greater selectivity to oligonucleotides and DNA.

The reliable study of ASO and siRNA in biological samples requires a selective and reproducible extraction method. The most commonly used methods for DNA, RNA, and antisense oligonucleotides are liquid-liquid extraction, enzymatic protein digestion, solid phase extraction, and hybridization. These methods have many drawbacks. The selectivity needs to be increased in isolating metabolites from serum and cerebrospinal fluid, as it is a critical step and a limitation of currently used methods. Hence, the synthesis and application of new adsorbents for extracting oligonucleotides from biological samples need systematic and extensive experiments to develop new and improved methods.

1.3. Work plan

- Synthesis of new materials with different types of functional groups for the extraction of ASO and siRNA
- Instrumental characterization of synthesized materials.
- Application of newly synthesized materials for the extraction of ASO and siRNA from standard solutions and biological samples.
- Application of different chromatographic modes (SEC, AEC, HILIC, IP RP HPLC) for separation of ASO and siRNA mixtures
- Application of different detectors for the identification and quantification of ASO and siRNA
- Development of a final extraction, separation, identification, and determination procedure for ASO and siRNA in serum samples.

1.4. Literature *(max. 10 listed, as a suggestion for a PhD candidate)*

Ł. Nuckowski, A. Kaczmarkiewicz, S. Studzińska, Journal of Chromatography B, 1090 (2018) 90–100.
A. Kaczmarkiewicz, Ł. Nuckowski, S. Studzińska, B. Buszewski, Critical Reviews in Analytical Chemistry, 49 (2019) 256-270.
S. Studzińska, Talanta, 176 (2018) 329-343.
A. Kilanowska, S. Studzińska, RSC Advances, 10 (2020) 34501-34516.
S. Studzińska, M. Mazurkiewicz-Bełdzińska, B. Buszewski, International Journal of Molecular Sciences, 23 (2022) 10166.
S. Studzińska, K. Ostrowska, Z. Vosahlova, K. Sasim, Application of amino acid-based adsorbents for the extraction of antisense oligonucleotides from serum samples, Talanta, Talanta 298 (2026) 128973
S. Studzińska, A. Lemska, J. Szymarek, M. Mazurkiewicz-Bełdzińska, Therapeutic oligonucleotide (nusinersen) metabolism in cerebrospinal fluid samples of patients with spinal muscular atrophy based on liquid chromatography coupled with mass spectrometry data, Analytica Chimica Acta, 1404 (2026) 345445

1.5. Required initial knowledge and skills of the PhD candidate

1. university Master's degree in chemistry;
2. strong motivation for scientific work and an open mind, willingness to conduct scientific research;
3. authorship of publications and/or conference reports;
4. an additional advantage would be if the candidate could demonstrate honors awarded for scientific research, scholarships and prizes, participation in scientific workshops and training, participation in research projects;
5. knowledge of analytical chemistry, knowledge about advanced instrumental techniques, knowledge in the field of liquid chromatography and extraction techniques;

6. experience in working with oligonucleotides, DNA or pyridazine derivatives and/or separation techniques is welcome;
7. willingness to prepare a valuable dissertation in a short period;
8. knowledge of English necessary for independent scientific work (preparation of reports, scientific publications, participation in scientific internships, and conference presentations).

1.6. Expected development of the PhD candidate's knowledge and skills

Acquisition of the ability to synthesize and characterize chemically modified adsorbents for extraction and stationary phases for separation. The ability to independently develop extraction and chromatographic separation methods. The acquisition of the ability to write scientific papers.